REMARKS

Restriction/Election

Applicants confirm election of Group I, claims 1-14 and 16-18, with traverse. Claim 15 (Group III) is identical to Claim 14, except that claim 15 recites that the cell of claim 1 is in situ, whereas claim 14 recites that the cell of claim 1 is in vitro. The location or context of the same claimed cell should not be grounds for characterizing "separate and distinct" inventions. We request rejoinder of duly limited, dependent method claims 19-22 (Group II). Group IV and group V claims are canceled.

Amendments

The informality at p.8, line 24 has been corrected. This amendments merely spells out the abbreviated singular and plural forms, and introduces no new matter.

The cited "etc., etc." at p.8, line 30 is submitted to be correct as written. The contextual sentence notes that the heterologous protein domain may be (1) a target protein to be labeled or traced, (2) a second labeled domain such as a second fluorescent domain, which may be provided by (i) a different phycobiliprotein domain, (ii) a phytochrome domain, (iii) a GFP domain, (iv) etc., (3) etc. Hence, the first "etc." is in reference to the list of heterologous protein domains, and the second "etc." is in reference to the list of fluorescent domains.

35USC112, second paragraph

The claims are submitted to be definite as written.

In claim 1, the recited heterologous fusion protein is the displayed domain as described inter alia, on p.7, line 19 - p.10, line 8.

In claim 1, the cell "makes and comprises" all the recited components: a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase.

Claim 1 is not incomplete, as the required bilin must be present in the cell. Dependent claims 2-4 require that the required bilin be formed by a reaction in the cell between a resident

heme and a resident heme oxygenase.

Claims 5-9 require that the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain. The claims do not require any particular difference in fluorescence, nor are they dependent on operator or equipment variation. So long as the heterologous protein domain is spectroscopically distinguishable from the first holo-phycobiliprotein domain, the limitation is met.

Claims 13-14 have been amended as suggested, and claim 15 similarly.

Claim 17 has been amended to cancel the inherent "bound".

We note the proposed revised claim 1 appears to combine the limitations of pending claims 1 and 6. However, the Specification thoroughly teaches heterologous protein domains (displayed domains) that are neither fluorescent nor comprise a second holophycobiliprotein domain. See, inter alia, p.7, line 19 - p.10, line 8.

35USC103(a)

The field of the invention is recombinant cells engineered to express heterologous holo-phycobiliproteins. Our claims require a recombinant cell comprising a reconstituted holophycobiliprotein biosynthetic pathway. In particular, (a) the required cell must express a holo-phycobiliprotein fusion protein comprising a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain fused to a heterologous protein domain, and (b) the required cell must make and comprise components: a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase, which components react inside the cell to form the holo-phycobiliprotein fusion protein.

Phycobiliproteins are a family of light-harvesting proteins found in cyanobacteria, red algae, and the cryptomonads. These proteins absorb strongly in the visible region of the spectrum because they carry various covalently attached linear tetrapyrrole prosthetic groups (bilins). Phycobiliproteins are tightly associated $\alpha\beta$ heterodimers, in which each subunit carries bilin(s) thioether-linked to particular cysteinyl residues. Prior to the present invention, holophycobiliprotein expression was limited to the cyanobacteria, red algae and cryptomonads

that naturally express these proteins, severely limiting their commercial application. Various steps involved in bilin biosynthesis and bilin addition to apophycobiliprotein subunits had been inferred, but never had anyone disclosed or suggested engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway as claimed.

The present invention teaches how to engineer cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway, allowing recombinant protein expression in more convenient cellular expression systems. Shortly after our filing date, we published descriptions of our reconstitution of two distinct holophycobiliprotein biosynthetic pathways in heterologous cells (Tooley, Cai and Glazer, Proc. Natl. Acad. Sci. USA. 2001 Sept 11, 98 (19), 10560–65; and Tooley and Glazer, J Bacteriol, Sept 2002, 184 (17), 4666-4671). In addition, following the filing and publication of our protocols, two other laboratories reported analogous reconstitution of biosynthetic pathways for holophytochromes (the cited Landgraf et al., 2001, FEBS Letters 508, 459-62, and Gambetta and Lagarias Proc. Natl. Acad. Sci. USA. 2001 Sept 11, 98 (19), 10566–71). Phytochromes are biliprotein photoreceptors found in plants and chlorophyll-containing prokaryotes. Like the light-harvesting phycobiliproteins, phytochromes possess thioether-linked linear tetrapyrrole (bilin) prosthetic groups that enable them to absorb visible light.

The Applicants are intimately familiar with the cited Fairchild (1992, PNAS 89:7017-21), Allnutt (US Pat/PUB No. 20010055783, June 16, 2001) and Frankenberg (2001, The Plant Cell 13:965-78) references. Fairchild describes in vitro phycocyanobilin lyase activity. The investigators show that a crude and fractionated cyanobacterial extract can catalyze bilin addition to the alpha subunit of apophycocyanin (apo-αPC), and that recombinant subunits of the heterodimeric phycocyanobilin lyase, the recombinant CpCE and CpcF proteins, enhanced addition (e.g. Fig. 2). They also show that cyanobacteria-expressed holophycocyanin could serve as a bilin donor in these reactions (e.g. Fig. 3). Nowhere does this reference teach or suggest that a holophycobiliprotein could be expressed in any cell other than a cyanobacterium that naturally expresses such proteins. Fairchild does not teach or suggest modifying E. coli to make a holophycobiliprotein. Fairchild does express apo-phycobiliproteins in E. coli, but when they need a cell to make the holo-form, they exclusively teach expression in cyanobacteria (e.g.

p.7018, col.2, lines 36-62).

As explained in our Specification, Fairchild is one of several early reports which inferred steps involved in bilin biosynthesis and bilin addition to apophycobiliprotein subunits in cyanobacteria (Specification, p.1, lines 21-22). However, none of these reports teach or suggest the feasibility of engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway.

Allnutt describes using developed methods for manipulating cyanobacteria to express phycobiliprotein fusion proteins. Because there was no other way of making holophycobiliproteins at the time, Allnutt describes expressing these proteins exclusively in cyanobacteria (e.g. Allnutt Examples 1-12). Nowhere does this reference teach or suggest that a holophycobiliprotein could be expressed in any cell other than a cyanobacterium that naturally expresses such proteins, but like Farchild, when Allnutt needs a cell to make a holophycobiliprotein, they exclusively teach expression in cyanobacteria.

Frankenberg (2001, The Plant Cell 13:965-78) identified genes encoding bilin reductases that catalyze the ferredoxin-dependent reduction of biliverdin IXα to phytobilins. As explained in our Specification, Frankenberg is one of several early reports which inferred steps involved in bilin biosynthesis and bilin addition to apophycobiliprotein subunits in cyanobacteria (Specification, p.1, lines 21-22). Of course, these reports provide necessary background information on enzymes that might ultimately prove necessary to reconstitute a holophycobiliprotein biosynthetic pathway. However, simply disclosing that this or that enzyme may be involved in the natural holophycobiliprotein biosynthetic pathway does not teach or suggest the feasibility of engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway.

As noted above, our claims require a recombinant cell comprising a reconstituted holophycobiliprotein biosynthetic pathway. The required cell must (a) express a holo-phycobiliprotein fusion protein comprising a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain fused to a heterologous protein domain, and (b) make and comprise components: a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a

recombinant phycobiliprotein domain-bilin lyase, which components react inside the cell to form the holo-phycobiliprotein fusion protein. Prior to our disclosure, no one had disclosed or suggested engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway as claimed.

The Examiner is invited to call the undersigned if she would like to amend the claims to clarify the foregoing or seeks further clarification of the claim language.

We petition for and authorize charging our Deposit Account No.19-0750 all necessary extensions of time. The Commissioner is authorized to charge any fees or credit any overcharges relating to this communication to our Dep. Acct. No.19-0750 (order B01-114-1).

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP

Richard Aron Osman, J.D., Ph.D., Reg. No. 36,627
Tel: (650) 343-4341; Fax: (650)343-4342

"To Help Our Customers Get Patents"
Mission Statement, USPTO External Customer Services Guide